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Tumors are unique organs defined by abnormal signaling and context

Derek Radisky, Carmen Hagios and Mina J. Bissell *

Many cancer investigations have focussed on the eradication of the cancer cell itself and in doing so, overlook the inherent complexity and heterogeneity of solid tumors. Here, we argue that, in many cases, it is the altered communication within the tumor, rather than mutations per se*, that is the defining characteristic of cancer. As a result, tumorigenesis can be indirectly initiated by environmental or inherited factors that affect the stromal cells. We propose that anticancer research might be more effective if aimed at eradicating the cause of abnormality rather than just treating the end result.*

Key words: epithelial–stromal interactions / microenvironment / tumor progression

Introduction

Many of the current models of multistage carcinogenesis describe cancer in a cell-autonomous fashion, as a progression of genetic mutations in an increasingly deranged tumor cell mass.[1] These models have been enormously successful in the identification and characterization of many of the tumor suppressor genes and potential oncogenes that are involved in cancer susceptibility.[2] It is equally important, however, to recognize that solid tumors are more than just a clonal expansion of renegade mutant cells, but are instead heterogeneous and structurally complex.[3] In this review, we present the hypothesis that tumor progression may be best appreciated as a product of the evolving interactions between the different cell types within the tumor and the microenvironment. For the purposes of this discussion we will use the terms 'tumor' and 'cancer' not in their conventional sense, as a description of a monoclonal expansion of cells, but rather to encompass the many cell types contained within the tumor. In this larger framework, tumor progression can be considered a developmental process, in which a complex, multicellular organ forms in response to signaling between different cell types. It is one of the most important goals of this review to argue that this viewpoint, of tumors as organs, is completely consistent with the current body of cancer research.

We will begin with a summary of some of the mechanisms for communication between the cells that comprise the tumor. Just as the function of normal organs is determined by reciprocal communication between the cells in the epithelial layer and in the surrounding stroma, so the same organizational principle applies to cancer, and the progression from metaplasia to malignancy can be characterized by the increasingly abnormal communications between the cells that comprise the tumor and their microenvironment. The tumor responds to these changing interactions, and as it evolves, it acquires nutrients, it evades natural anticancer mechanisms, and eventually, it invades neighboring tissues.[4,5] In traditional models, these changes have been assumed to result solely from pre-existing or acquired defects in the core epithelial cells. While this may be the case in some instances, here we will present examples in which the changes are initiated by factors that directly affect stromal cells, and in these cases, the altered stroma itself becomes the tumorigenic agent. These observations suggest that disruptive stresses may contribute to the development of cancer and that treatments to restore normal communication between the different cell types within a tumor could be effective in combating cancer. We will present several recently developed experimental systems that can be used to investigate the cellular interactions

*From the Life Science Division, Lawrence Berkeley National Laboratory, University of California, Berkeley, CA 94720, USA. *Corresponding author. E-mail:* mjbissell@lbl.gov

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in developing tumors, and more importantly, to determine how to manipulate these interactions to produce a functional reversion of the tumorigenic phenotype.

Communications between cells define both normal and tumor function

The cellular microenvironment provides functional context

Cells are surrounded by a complex, three-dimensional extracellular matrix (ECM) that contains a mixture of glycoproteins, proteoglycans, cytokines, and growth factors.[6,7] ECM provides both structural support and contextual information for cells to determine the correct response to a given set of stimuli.[8] The composition of ECM varies considerably both between and within different tissues,[9,10] and ECM changes temporally, as an adaptation to changing signals during normal developmental processes.[11,12]

Each cell type displays a unique array of surface receptors tuned to its natural tissue environment. An enormous body of literature has identified integrins as the principal mediators of this interaction;[13,14] more recently, a number of other important cell surface ECM receptors have been identified, although their roles are not yet as well characterized.[13,15,16] Ligation of cell surface receptors to ECM changes cell shape and behavior, alters the binding affinity or cellular distribution of other integrin and non-integrin receptors,[17] and profoundly influences the response of the cell to soluble molecules such as growth factors.[18] Depending upon the ECM context, the same soluble factors may alternatively cause cells to functionally differentiate, to initiate proliferation, to arrest growth, or to cause apoptotic cell death.[19] Control of growth factor signaling by ECM-defined context ensures that cells divide and differentiate only as needed by the organism.

Basement membrane (BM), a specialized form of ECM, separates epithelial and stromal cells,[20,21] and as 90% of all tumors involve epithelial cell proliferation, changes in the BM are a particular focus of cancer investigations. Epithelial BM is normally composed of laminins, collagen type IV, nidogen, and proteoglycans such as perlecan,[22,23] and each of these components have multiple isoforms. For example, combinations of different subunits in the laminin trimer can produce 11 variants, each unique in structure, localization and biological activity.[22] Similarly, collagen IV can have multiple isoforms,[24,25] and two forms of nidogen have been identified.[26] Investigations of ECM composition are starting to reveal a picture of exquisite microenvironmental control[27,28] with considerable influence on developmental processes.[29] For example, transgenic animals lacking only one isotype of an ECM protein can develop with catastrophic defects in a single tissue, although the rest of the animal is apparently unaffected.[30,31]

Cancer as a disruption of ECM control

Normal homeostasis is maintained by interpreting growth factor signaling in the ECM context. This control can be lost through the breakdown of communication mechanisms between the epithelium and the surrounding stroma (Figure 1). For example, an epithelial cell might incorrectly initiate a signal to the stroma resulting in the stromal production of a growth factor that, in turn, can stimulate the incorrect proliferation of neighboring epithelial cells.[32] Alternatively, an aberrant matrix component produced by stromal cells in response to a local stress might be perceived by neighboring cells as a signal to grow or to enter a new developmental pathway.[33,34] Under normal homeostasis, these mistakes will be corrected by cell cycle arrest or apoptotic cell death. Occasionally, if the abnormal signal persists, the behavior can become increasingly unbalanced,[35] creating a growing, interdependent, heterogeneous tissue, defined as a tumor by its ability to grow and by its unresponsiveness to normal physiological controls.[36,37]

As the tumor grows, the cells within continue to respond to their immediate environment,[38] and since the tumor is itself in a state of flux, multiple and conflicting signals can lead to increased complexity and heterozygosity.[39,40] Tumor remodeling can include interactions with alternative ECM components such as tenascin, fibronectin, and isoforms of laminin,[41,42] alterations that can produce cellular proliferation, structural disruption,[43,44] and circumvention of apoptosis.[45] Throughout the process of tumor development, the composition of ECM is controlled in a reciprocal manner between epithelial and stromal cells,[46,47] and it is through these reciprocal interactions that the tumor creates a microenvironment that is favorable to proliferation,[48,49] recruits new blood vessels,[50,51] and stimulates the production of metalloproteases to invade adjacent tissues.[52,53]

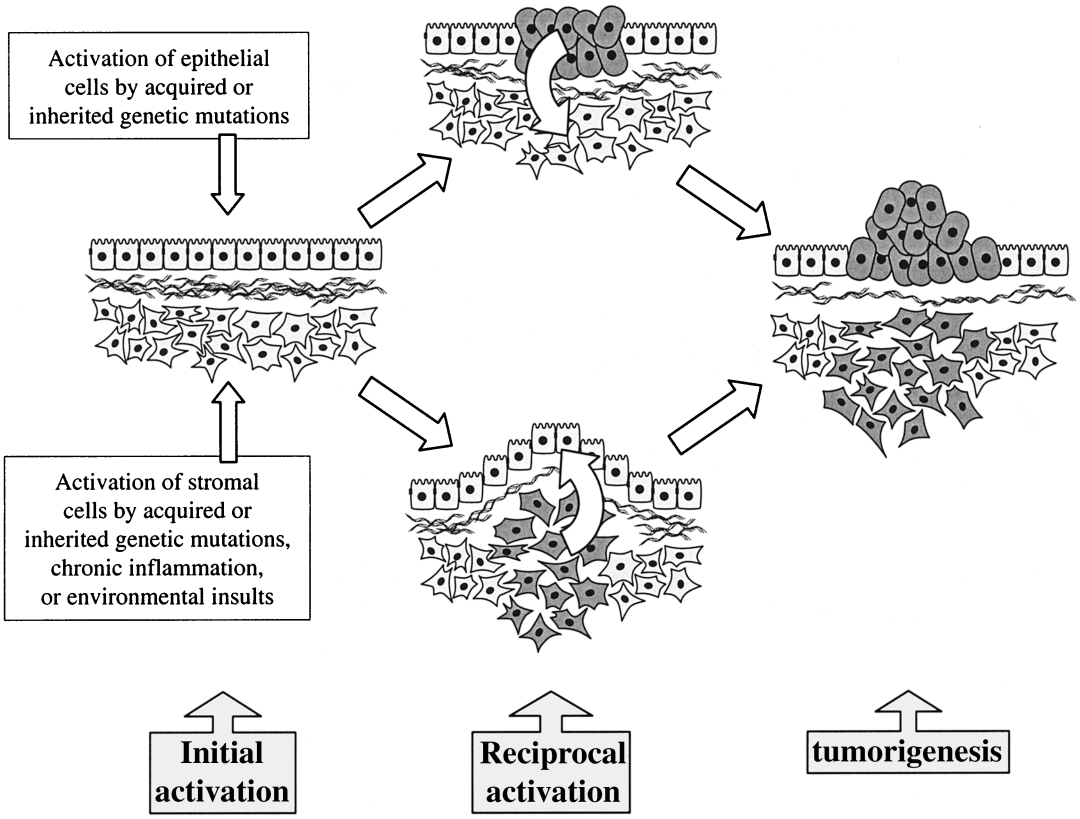

Figure 1. Tumor production can be the product of epithelial mutations that create an expanding, reactive tumor. However, it is also possible for mutations or environmental factors to create a reactive stroma in advance of epithelial mutations, and in turn produce an unstable epithelium to cause tumorigenesis.

Aberrant cell–cell signaling can be tumorigenic

Factors that are not mutagenic *per se* may still cause cancer by promoting tumorigenic developmental programs.[54,55] This situation occurs from sustained disruptions of signaling between the epithelium and the stroma. Such disruptions may occur in cases of chronic inflammation, from defects that lead to stromal dysfunction, and events associated with aging.

Inflammation

The relationship between tissue disruption and *de novo* carcinogenesis has been best characterized in association with sites of chronic inflammation.[56] Persistent infections with *Helicobacter pylori* cause both gastric cancer[57,58] and gastric lymphoma,[59] and anti-*Helicobacter* therapy can produce tumorigenic reversion.[60] Similarly, hepatitis C infections produce hepatocellular carcinoma[61,62] and schistosoiasis is a major cause of bladder cancer.[63]

Chronic exposure to damaging agents that induce persistent inflammation may also result in cancer: for example, reflux esophagitis can cause esophageal cancer,[64,65] chronic pancreatitis can lead to pancreatic cancer,[66,67] and ulcerative colitis (UC) can produce colorectal carcinoma.[68,69] In many of these cases, excessive proliferation and genetic damage in the stromal compartment can be detected even before the induction of malignant carcinoma.[70] Hepatic fibrosis can lead to liver carcinoma,[71] fibrotic breast disease can predispose to breast cancer,[72] and environmentally-induced fibrotic disorders of the lung can increase incidence of lung cancer.[73,74] The connection between inflammation and cancer is also reflected in epidemiological studies in which anti-inflammatory inhibitors of the enzyme cyclooxygenase-2 act as chemopreventative agents.[75]

The role of stroma

Phenotypic defects have been observed in cells cultured from tissues remote from the primary tumors. Studies performed more than a decade ago on skin fibroblasts from breast cancer patients found significant differences relative to fibroblasts from unaffected individuals, including abnormal migratory patterns on collagen gels,[76,77] abnormal actin distribution,[78] decreased anchorage-dependent growth, increased colony formation, and loss of cell cycle controls.[79,80] These differences were at times striking, and in several cases, detection of the cell growth defects preceded the discovery of the malignancy.[79,80]

The existence of mutations in the tumor stroma has also been documented. This phenomenon defines a category of tumor suppressor genes that, when mutated or functionally absent, can disrupt stromal function and thus produce a tumorigenic microenvironment for the epithelial compartment.[81] Heritable genetic defects that affect the stroma can produce carcinoma in juvenile polyposis syndrome.[82,83] Similar effects have also been observed in syndromes associated with increased endometrial polyps.[84] This effect may also contribute to breast cancer, as analyses of cells adjacent to mammary tumors have found genetic alterations in the stroma that apparently preceded genotypic changes in the epithelial carcinomas.[85,86] Taken together, these observations suggest that systemic alterations in intercellular communication may precede and be a causal factor in the development of localized tumors.

Experimentally induced alterations

Targeted expression of the MMP stromelysin-1 (SL-1) in the mammary epithelium of tansgenic mice[87] produced spontaneous acquisition of stromal features characteristic of neoplastic states[88] that eventually led to full malignancy.[89–91] Analysis of the transgenic mice revealed that SL-1, expressed initially at low levels in the mammary epithelial cells, was subsequently produced at much higher levels in stromal fibroblasts (Figure 2).[88] This observation suggested a feedback loop for SL-1 expression between the epithelium and the stroma, and consistent with this hypothesis, cultured epithelial cells that contained inducible SL-1[92,93] also formed tumors that became independent of transgene expression.[90] Increased expression of the metalloproteinases matrilysin[94] and stromelysin-3[95,96] has also been characterized as a feedback mechanism for maintaining the tumorigenic state.

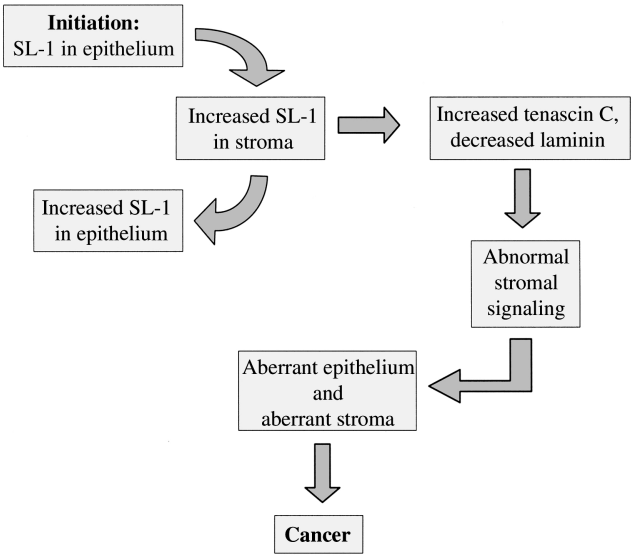

Figure 2. Induction of stromelysin-1 in mammary epithelial cells led to greatly increased production in surrounding fibroblasts, disorganization and destruction of the BM, incorrect signaling between the stroma and epithelium, and subsequent neoplasia.

Normal ECM can inhibit tumor initiation and progression

As abnormal ECM signaling can direct functional epithelial cells toward neoplasia, normalized signaling can arrest or even revert the malignant behavior of tumors. This phenomenon is found in the mouse skin carcinogenesis model, in which mice treated with mutagenic agents that activate H-ras do not develop tumors until after the application of compounds that induce TGF-β.[97] Here, activating mutations are insufficient to override normal growth controls. Although many details of this anticancer mechanism are unknown, its significance is indisputable, as spontaneous, ras-activating mutations are predicted to occur in thousands of cells per day,[98] yet ras-related skin cancer is a relatively rare event. If transforming mutations can be suppressed by normal microenvironmental controls, then potentially carcinogenic mutations could be more frequent than the occurrence of outright malignancy. In validation of this prediction, analysis of morphologically normal epithelial cells adjacent to breast tumors has revealed a number of chromosomal rearrangements,[99–101] some of which were also found in the tumors.

Coculture experiments have been used to investigate the mechanisms that guide tumor–stroma

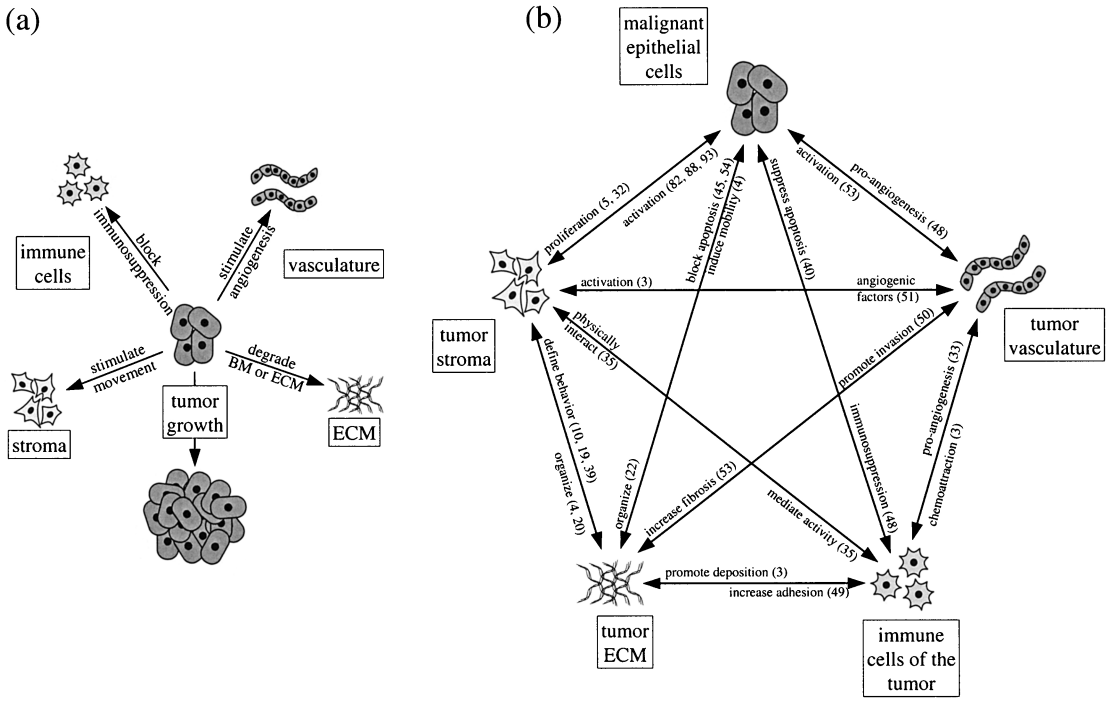

Figure 3. (a) Cell autonomous. Early models depicted a mass of deranged, monoclonal cells that produced the cancer phenotype through manipulation of surrounding cellular structures. (b) Multicellular. More accurate models account for the interconnected nature of tumors. Here, some of the interactive effects between the components of the tumor are listed, along with references from the text.

interactions. Fibroblasts from normal breasts have been found to inhibit the growth of nontumorigenic mammary epithelial cells, although fibroblasts from cancerous breasts stimulated proliferation;[102,103] similar results have been found in coculture experiments with prostate cells.[5,104] We now need to understand the mechanisms involved in these interactions.

Models for investigating epithelial–stromal interactions

Observations that abnormal signaling can promote tumorigenesis and normal signaling can block abnormal growth suggest that early stage tumors retain some responsiveness to ECM-mediated growth controls. Our understanding of tumor characteristics at these early stages is primitive, however. To better study the mechanisms of formation, we have devised an assay that models the breast terminal duct lobule in a three-dimensional, reconstituted basement membrane (rBM).[105] The mammary epithelial cells used in the assay were derived from a reduction mammoplasty[106] and repeatedly passaged until they had acquired numerous chromosomal abnormalities[107–109] and tumorigenicity.[110] Although the early passage (S1) cells were similar in appearance to the late passage (T4-2) cells when cultured on plastic substrate, the phenotypic difference was striking in rBM.[111] Under these conditions, the S1 cells arrested growth and formed a polarized, alveolar structure, while the T4-2 cells proliferated amorphously. Anti-integrin antibody treatment of the T4-2 cells to normalize the perception of the ECM effected reversion of both morphologic and tumorigenic properties.[112] Further investigations with this system have identified other processes that can inhibit malignancy.[113–115]

Organotypic models for other tissues have simulated stratified skin epithelium, characterized double paracrine signaling mechanisms[116,117] and identified normal environmental signals that can block malignant proliferation of tumorigenic cells.[118,119] Studies such as these provide indications of the potential for nondestructive intervention of early malignancies.

Conclusions

Many investigations of tumorigenesis and tumor progression have examined isolated facets of the cancer phenotype [Figure 3(a)]. These may be cell autonomous (such as uncontrolled DNA replication, loss of p53, or enhanced telomerase activity), or may involve incorrect tissue behavior (such as breakdown of cell–cell and cell–ECM contacts, incorrect expression of metalloproteases, or angiogenesis). This reductionist approach initially produced significant success in the development of effective cancer treatments, but recent progress has been less encouraging.[120]

In this review, we have presented a different view of a tumor as an interconnected and functional tissue [Figure 3(b)], albeit one with a singularly destructive effect on the greater organism. We suggest that the individual facets of tumor behavior are not experimentally separable, and that a complete understanding of the developmental program of tumors will require investigative approaches that account for this inherent interdependence. To begin these investigations, we have devised an assay that models a simplified mammary tissue, and then followed the changes associated with acquisition of malignancy. Other models are under development, but much more work is required. We need to understand tumor signaling at the critical early stages of development in much greater detail, and we need to apply this information to more sophisticated culture systems that can model both normal and tumor tissues. These approaches are still in their infancy, but we believe that they will be essential in developing the next, and hopefully more effective, stage of anticancer treatments.

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